lies below 0°; in the case of the alkalies our own experiments have shown that the curves, which have usually been represented as straight lines or flat parabolas, all exhibit inflexions, in the case of the alkalies, LiOH, NaOH and KOH at about 40°, and in the case of the alkaline earths, $Ca(OH)_2$, $Sr(OH)_2$ and $Ba(OH)_2$, at about 25°; in the case of the less highly ionised salts, such as magnesium sulphate, the inflexion also lies below 100°, but in the case of salts such as potassium chloride, which have a high coefficient of ionisation in solution and are also electrolytes *per se* in the fused state, the inflexion lies above the boiling-point of the solution.]

"On the Measurement of the Bactericidal Power of Small Samples of Blood under Aerobic and Anaerobic Conditions, and on the Comparative Bactericidal Effect of Human Blood drawn off and tested under these Contrasted Conditions." By A. E. WRIGHT, M.D., Professor of Pathology, Army Medical School, Netley. Communicated by Professor J. R. BRADFORD, F.R.S. Received April 7, 1902. Received in revised form August 5, 1902.

SECTION I.—*Method of Measuring the Bactericidal Power of the Blood under Ordinary (Aerobic) Conditions.*

As a preliminary to placing on record certain observations made in connection with the bactericidal power of human blood, I propose to describe in detail the technique which has been elaborated by me with a view to carrying out these and similar investigations.

A measurement of the bactericidal power of the blood involves in the first place a standardisation of bacterial culture employed.

I. *Standardisation of the Bacterial Culture employed.*

A standardisation which would seem to satisfy all practical requirements can be achieved (*a*) by employing in the course of a series of experiments one and the same stock of bacteria; (*b*) by employing in each experiment a young (*e.g.*, a 24 hour old) culture; (*c*) by determining in each case the number of living bacteria contained in a measured volume of that culture.

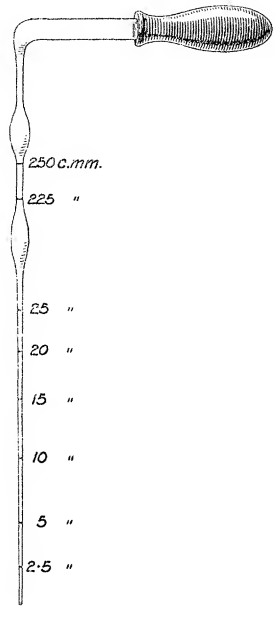
The determination last mentioned* involves—*first*, the making of a

* (Added 3.8.02.) Where, in lieu of the number of living bacteria, the total number of bacteria in a culture is to be determined, this can be directly determined under the microscope by the method I have described in the 'Lancet' of July 5, 1902.

measured dilution of the culture (in the case of an ordinary bacterial culture, dilutions of 1,000,000 fold to 10,000,000 fold are appropriate), and, *secondly*, the transference—with a view to the subsequent enumeration of the colonies which develop—of a series of measured volumes of the diluted culture to the surface of a solid nutrient medium.

The processes of diluting and measuring off the desired volumes of diluted culture can be conveniently and unlaboriously carried out by means of the diluting pipette figured below. (Fig. 1).

FIG. 1.



(1.) *Method of making and calibrating the "Diluting Pipette."*

(*a.*) A piece of glass tubing about 15 cm. long is drawn out at one end into a capillary stem.

(*b.*) A standard 5-cm. pipette is fitted with a rubber teat,* and is then filled in up to the calibration mark with mercury. The 5 cm. of mercury is now transferred from the capillary pipette to the wide end of the glass tube, and is made to enter the upper portion of the capillary stem.

(*c.*) When this has been effected, the points corresponding to the upper and lower ends of the mercury column are marked off on the outside of the capillary stem with a coloured pencil, preferably one of the oil pencils sold for writing on glass.

(*d.*) The mercury column is now displaced downwards until the upper end of the column stands opposite the lower of the calibration marks. This point is again marked off with the coloured pencil.

(*e.*) By a similar procedure, three more marks indicating divisions of similar value are placed on the outside of the capillary stem.

(*f.*) This done, the tube is filed and broken off at the lowest mark.

(*g.*) It will now be convenient to divide the lowest 5-cm. division into two divisions of 2·5 cm. This can be conveniently effected by the following method of trial and error :—Mercury is drawn up into the tube until the upper limit of the column of metal stands as nearly as possible midway between the orifice and the first 5-cm. division mark. The point corresponding to the proximal end of the mercury column

* A mechanically controlled teat, such as that made by Mr. A. E. Dean, jun., 73, Hatton Garden, E.C., is a convenient form to employ for this purpose.

may now be tentatively indicated on the outside of the capillary stem by a light pencil mark. This done, the column of mercury is displaced until its proximal end stands level with the 5-cm. division mark. If the distal end of the mercury column now coincides with the tentative subdivision mark, this last gives the desired 2·5-cm. division. If it does not coincide, the desired point will be situated half-way between the point now indicated by the proximal extremity of the mercury column and the point indicated by the original trial subdivision mark.

(*h.*) What has been achieved up to this point is a graduation of the capillary stem into five divisions of 5 cm., and a subdivision of the first of these into two 2·5-cm. divisions. A further process of graduation in terms of 25 cm. is now taken in hand, with a view to finding the points corresponding respectively to 225 and 250 cm.

(*i.*) For this purpose a rubber teat is placed upon the upper end of the tube, and a negative pressure having been established, the capillary stem is filled up to the 25-cm. mark with mercury, water, or a coloured fluid. It is then filled in succession with eight further 25-cm. volumes, the 25-cm. volumes being in each case spaced off from each other by a bubble of air. After these air bubbles have risen to the surface in the wide upper portion of the tube, and the separate volumes have here united to form a single body of fluid, a mark is placed on the outside of the tube to indicate the 225-cm. point. An additional 25-cm. volume of fluid is now introduced, and the point corresponding to 250 cm. is similarly registered.

These last marks, be it noted, serve only for the provisional graduation of the tube.

(*j.*) With a view to achieving a more accurate graduation, the portion of the glass tube between the 225 and 250 cm. graduation marks is fused in the blow-pipe flame, and is drawn out into a short thick capillary tube such as will admit of a more accurate calibration.

(*k.*) The calibration in terms of 25 cm. is now repeated, and the points corresponding to 9 and 10 multiples of 25 cm. are now finally marked off on the narrow portion of the tube.

(*l.*) A safety chamber is formed on the upper part of the tube, the wide end of this last being carried round at right angles to the stem to allow of more convenient manipulation.

(*m.*) Lastly, the pencil marks are carried round the whole circumference of the capillary tube, and they are fixed upon the glass by passing them through the flame.

(2.) *Method of employing the Capillary-diluting Pipette.*

By means of a diluting pipette fitted with a rubber teat any desired dilution of the culture can be obtained very unlaboriously.

A *ten-fold dilution* of the culture—the dilution which is perhaps most often required—is made by taking first 25 cm. of the culture, and then, after the interposition of an air bubble, filling up to the 250-cm. mark with sterile broth. It can also, and this avoids any contamination of the sterile diluting fluid, be made by filling up first with 225 cm. of the broth, and completing up to the 250-cm. mark with the culture.

A *six-fold dilution*, should such be required, would be obtained by filling in to the 250-cm. mark with sterile broth, and then completing with two volumes of culture, these last being isolated as before by intervening air bubbles.

A *five-fold dilution* is obtained by filling in with two separate 25-cm. volumes of the culture, and completing up to the 250-cm. mark with sterile broth.

A *two and a half-fold dilution* would be obtained by filling in with four separate volumes of 25 cm., and completing up to the 250-cm. mark with sterile broth.

Dilutions of a different order can be obtained by filling in the pipette as occasions may require with 2·5 or 5 cm. of culture, and then completing to 250 cm. with sterile broth. By this means dilutions of 1 in 100 and 1 in 50 respectively can be obtained *uno saltu*.

By a series of successive dilutions, made in each case after washing out the pipette with boiling sterile water, any desired attenuation of the culture can be quickly arrived at. The dilution of 1 in 1,000,000 ordinarily required for the purposes of enumeration will be obtained by three successive dilutions of 1 in 100.

(3.) *Method of Eliciting the Number of Micro-organisms contained in the Diluted Culture.*

The required dilution of, let us say, 1 in 1,000,000 having been prepared, the pipette would, after sterilisation in boiling sterile water, be filled in with, say, three successive 10 cm. volumes of the diluted culture. A corresponding number of agar tubes having been taken in hand, the three 10 cm. volumes of diluted culture would be separately transferred to the surface of the nutrient medium, care being taken in each case to spread out the fluid over as large an area of surface as possible.

After incubation, the number of bacteria in each 10 cm. volumes of diluted culture would be deduced from the number of colonies which develop on the corresponding agar tube.

After averaging the number of bacteria contained in the three tubes, the number of living bacteria contained in 1 c.c. of the original culture would be found by a simple arithmetical process.

II. *Procedures in connection with the actual carrying out of the Bactericidal Estimation.*

In connection with the actual carrying out of the bactericidal estimation, we have to consider :—

(1.) The collection of the sample of blood for examination.

(2.) The preparation of a graduated series of dilutions of the bacterial culture.

(3.) The special form of capillary-testing pipette required for the subsequent procedures.

(4.) The method of employing the testing pipette just mentioned, *i.e.*, the method of mixing a series of measured volumes of serum with in each case an equal volume of the successive bacterial dilutions, and the method of determining the sterility or otherwise of the mixtures after the serum has acted upon the bacteria for an appropriate period.

(1.) *Collection of the Sample of Blood.*

The quantity of blood required for an ordinary bactericidal estimation need never exceed 1 c.c.* Much more than the quantity required can, in the case of man, readily be obtained by driving the blood into the pulp of the finger by winding a handkerchief round the digit, making a prick with a needle or spicule of glass, and then making pressure on the pulp.

A convenient form of blood capsule is that figured below (fig. 2). The upper end of capsule, when drawn out in a peep flame or in the flame of a lucifer match, provides an aseptic pricker. When proceeding to collect the blood both this (A) and the end of the curved limb (B) are broken off. The blood then flows into the capsule, as shown in fig. 3, under the combined action of gravity and capillarity. When

Fig. 2.

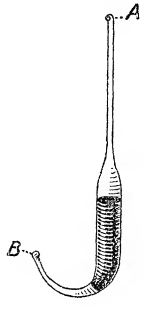
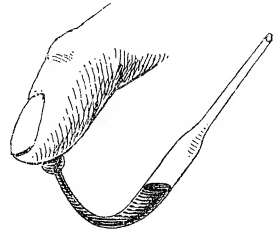


Fig. 3.



* Where only minimal amounts of blood are available, the difficulty can be got over either by the employing very fine capillary tubes or by mixing progressive dilutions of the serum with one and the same dilution of the bacterial culture.

sufficient blood has been collected, the upper portion of the capsule is gently warmed and the upper orifice is then immediately sealed up. As the air, which has been rarefied by warming, contracts, the blood is drawn up into the body of the capsule, leaving the orifice at (B) free for resealing. After the capsule has cooled, it is suspended by means of its curved arm in a hand centrifuge, and the blood is driven down by a few turns of the handle into a previously upper half of the capsule. It is now left at rest for a few minutes. When the serum begins to exude, the centrifuge is again brought into action.

When the estimation is to be taken in hand, the neck of the curved bulb of the capsule is sterilised in the flame and is cut across with a stout pair of bone forceps, which has been sterilised in the same manner.

Two further points in connection with sample of blood may appropriately be considered. The first of these relates to the question of the necessity for aseptic precautions in drawing off the blood. The second to the question of the interval which may elapse between the drawing off of the blood and the bactericidal estimation.

The blood should be drawn off with aseptic precautions. For this purpose the surface of the finger may be readily and effectually sterilised by moistening it with alcohol, and burning this off.

Where results which are comparable among themselves are desired, the bactericidal estimations ought, in all cases, to be undertaken within a very few hours after the samples of blood have been withdrawn. Where samples of blood are tested immediately after withdrawal, and again after an interval of 24 hours, it is usual to find a notable diminution of bactericidal power in the second estimation.

(2.) *Preparation of a Series of Graduated Dilutions of the Bacterial Culture.*

It has already been indicated, but it will be well at this point clearly to bring out the fact, that in the method of bactericidal estimation here described, a series of measured volumes of undiluted serum are brought in contact with a series of graduated dilutions of the culture, the object being to determine what is the lowest dilution of the culture with which a complete bactericidal effect is exerted.

The graduated dilutions of the culture which are required for this purpose, so far as they have not already been provided by the procedure undertaken in connection with the enumeration of the bacterial culture, would, at this stage, be prepared by the aid of the diluting pipette. I have found it convenient in the case of the typhoid bacillus to employ, in addition to the undiluted culture, in each case a 2 fold, 5 fold, 10 fold, 25 fold, 50 fold, 100 fold, 1000 fold, 10,000 fold, and 100,000 fold dilution.

The dilutions are to be held ready for use in a series of covered sterile watch-glasses.

(3.) *Description of the Special Form of Capillary Testing Pipette Employed in the Connection with the Method of Bactericidal Estimation here described.*

Fig. 4 shows the particular form of capillary testing pipette which has been found most suitable. The stem A—it will be noted that it is provided with a pencil mark—serves on the one hand as a measuring pipette for measuring off equivalent volumes of serum and bacterial culture, and on the other hand as a receptacle for the combined volumes of fluid during the period allotted to the action of the serum upon the culture.

FIG. 4.

The bulb B functions, in the first stage of the procedure described below, as a receptacle for the sterile broth afterwards used for testing the continued vitality or otherwise of the bacteria which have been exposed to the influence of the serum. In the second part of the procedure, the bulb of the pipette comes into use as a cultivation chamber. The bulb may conveniently possess a capacity of about 1 c.c. The spiral C* serves to prevent any access of contaminating bacteria to the interior of the bulb.

(4.) *Method of Employing the Capillary Testing Pipettes.*

The method of employing the testing pipettes is as follows:—

A mark with the coloured oil pencil having been placed upon the capillary stem at any convenient point, say at a point 1—1·5 cm. from the lower end, a rubber teat is fitted over the upper end of the tube.

The point of the capillary stem is now broken off between finger and thumb, the lower portion is sterilised in the flame, and the air is expelled from the teat.

Sterile broth, which has been placed ready to hand in a covered sterile watch-glass, is then aspirated into the pipette until the bulb is about two-thirds full.

The extremity of the capillary stem is now withdrawn from the

It will be found that the introduction of the spiral avoids the necessity for the troublesome plugging of the tube with cotton wool and the subsequent sterilisation process. The very simple trick of hand by which the spiral is made may be readily learned by imitating the motions associated with the making of a similar spiral upon a stiff cord or a very pliable wire.

broth, and the column of fluid which occupies it is allowed to run up very gently, so as to avoid any back lash, into the bulb. The inflow of air is arrested as soon as the capillary stem is empty of fluid.

(a.) *Method of Measuring off and Mixing together equal Volumes of Serum and Bacterial Cultures.*

The end of the capillary stem is now inserted into the narrow open end of the blood capsule, which has been placed ready to hand in a perforated rubber bung or other convenient receptacle. The serum is allowed to flow in until it reaches the pencil mark.

The orifice of the pipette is now raised above the surface of the serum, and a bubble of air is admitted into the tube to serve as an index for the next measurement.

This done, the end of the capillary stem is carried over into a watch-glass containing the particular dilution of the culture which is to be dealt with in this particular tube. The culture is allowed to flow in until the bubble of air has just been carried past the pencil mark.

The next procedure is to mix together the equal volumes of serum and culture which have been measured off. This is effected by blowing these two volumes out upon the surface of a sterile watch-glass—a pile of inverted sterile watch-glasses will for this purpose have been placed ready to hand—and drawing up and driving out the fluid several times in succession. After a little practice* this can be quite easily achieved without driving the sterile broth down from the bulb of the pipette into the lower part of the capillary stem and there contaminating it.

The column of mixed serum and culture is to be drawn up into the middle region of the capillary stem as a preliminary to sealing the lower end of the tube. It will be found that when the column is left in this position, the intervening column of air which occupies the upper portion of the capillary tube will effectually isolate the fluid in the bulb of the pipette for the mixture of serum and culture.

The teat is now removed, leaving the spiral to guard the contents of the tube against contamination, and the filling of the series of tubes with the remaining dilutions of the culture is proceeded with. When the whole series of tubes has been filled in, these are placed upright in a test tube labelled with the date and the source of the serum. The serum is then allowed to exert its influence on the bacteria with which it has been brought in contact for a fixed period at a fixed temperature.

* Until practice shall have conferred sufficient control over the teat, it will be advisable either to employ very fine capillary tubes or to provide a by-channel for the air by piercing the teat with a spicule of an extremely fine capillary tube.

I have found it convenient to allow the serum to remain in contact with the culture for a period of 18 to 24 hours at 37° C.

(b.) *Method of Testing the Continued Vitality or otherwise of the Bacteria which have been in Contact with the Serum.*

The sterile broth which has been filled into the capillary pipette furnishes, as we have seen, the means for determining whether the bacteria which have been brought in contact with the serum have or have not retained their vitality. If the serum has failed to kill the bacteria, this will be evidenced by the development of turbidity in the broth which will follow upon the aspiration of the column of fluid in the capillary stem into the bulb of the pipette. If, on the other hand, the serum has killed all the bacteria with which it has been mixed, the nutrient broth will, under the circumstances, remain clear.

The steps of the procedure are as follows:—

The tubes having been taken in hand singly, the lower portion of the capillary stem is in each case drawn out, and after heating in a peep-flame, into the finest possible filiform tube.

A condition of negative pressure is now established in the interior of the pipette by fitting over its upper end a collapsed rubber teat. While carefully regulating this negative pressure by keeping* the finger and thumb in position on the teat, the finely-drawn-out end of the capillary stem is gently snapped across. The column of fluid will then be very quietly carried up into the bulb of the pipette.

The determination of the continued sterility or otherwise of the broth may generally be made after incubation by mere naked eye inspection. Where a doubt arises either as to the existence of a growth, or as to the nature of the cultivation obtained, a drop of the culture may be microscopically examined or cultivated on nutrient agar.

III. *Method of Expressing the Results obtained by the Method of Bactericidal Estimation here in question.*

The question which is investigated by the method described above is, as has been seen, the question as to what is the lowest dilution of the particular enumerated culture employed which is completely sterilised by digestion with an equal volume of serum. No attempt is made to determine what reduction in number of living bacteria, and what subsequent increase occurs in the case of those tubes which are not completely sterilised.

It is claimed that by narrowing down the issue, as is here done, we escape from a fallacy which consistently arises in connection with estimations of bactericidal power arived at by a comparison of the

* *Vide* note on previous page.

results of bacterial enumerations carried out at a series of successive intervals upon one and the same mixture of serum and culture. The fallacy just referred to comes in in connection with the circumstance that all evidence of a bactericidal effect exerted will be obliterated if the intervals between the successive enumerations happen to be such as to allow of the covering up of losses due to the bactericidal action of the serum by a subsequent multiplication of the surviving micro-organisms.

A further point which has been kept in view in designing the above method, is the importance of obtaining a simple numerical expression for the bactericidal power of the blood.

Such a simple numerical expression is obtained by specifying the number of bacteria contained in 1 c.c. of the lowest dilution of the bacterial culture which is completely sterilised by digestion with an equal volume of serum.

While a convenient basis for the comparison of the bactericidal power of a series of different bloods is thus provided, it must be understood that the expression just referred to is nothing more than an arbitrary formula expressing the bactericidal effect of the serum brought into application in the form of a 50 per cent. solution.

If it is desired in any case to determine the bactericidal effect exerted by the serum in a practically undiluted condition, this can readily be achieved by making a graduated series of dilutions of the enumerated culture, using the serum itself as a diluent.

In concluding this section it will perhaps not be amiss to point out that the method of bactericidal estimation here described may be employed not only for determining the bactericidal power of the blood, but also for determining that of any chemical antiseptic.

Section II.—*Method of Measuring the Bactericidal Power of the Blood under Anaerobic Conditions.*

The method of measuring the bactericidal power of the blood under anaerobic conditions which is here to be described, is similar to the method described in the previous section, except in so far as the technique is modified with a view to excluding the air from contact with the blood.

Access of air is prevented by enveloping the blood in oil.

It is essential that this oil should be absolutely neutral, first, because the presence of fatty acid might affect the bactericidal power of the serum by diminishing its alkalinity and by precipitating its calcium salts, and, secondly, because an oil containing fatty acids is emulsified when it is brought in contact with serum, nutrient broth, and alkaline fluids generally. Such an emulsification would interfere with that sharp separation of the oil from the enclosed fluids which is absolutely

essential to the proper carrying out of the technique described below.

It will be well, therefore, to commence by describing the method adopted for the preparation of a fatty acid-free oil.

Method adopted for obtaining a Fatty acid-free Oil.

The method I have employed is a modification of that which was employed by the late Prof. E. Külz for obtaining a fatty acid-free oil for experiments in connection with pancreatic digestion.

The procedure will perhaps be most clearly described by detailing an actual experiment.

300 c.c. of a cheap variety of table oil (cotton oil ?)* was introduced into a litre flask along with 150 c.c. of half saturated barium hydrate solution. These fluids were digested together at 60° C. on a water bath for three hours, the contents of the flask being well shaken up at intervals.

After this time the contents had separated into three layers, an upper layer of more or less clear oil, a middle layer, about half an inch deep, of barium soaps, and a lower layer of barium hydrate solution.

A drop of the supernatant oil was now tested by shaking it up in a test tube with some 0·25 per cent. sodium carbonate solution. Indications of emulsification were quite absent.

The contents of the flask were now poured upon a wet filter. After the lapse of a few minutes, when the barium hydrate solution had filtered through, a clean dry beaker was placed under the funnel, and the whole filter stand was placed in a warm chamber. By next morning some 200 c.c. of clear oil were found in the beaker, the barium soaps having been left behind on the filter.

On shaking up the filtered oil with the sodium carbonate solution, it was found that this last showed a trace of turbidity. This turbidity was increased by breathing into the test tube and shaking up again.

In view of this, the whole volume of oil was now shaken up with distilled water, and a stream of carbonic acid gas was led through. The water and barium carbonate precipitate were then separated from the oil by filtration. When the oil thus purified was shaken up with the sodium carbonate solution, this last remained absolutely clear, the globules of oil remaining distinct and coming up promptly to the surface.

The fatty acid-free oil thus obtained is introduced into a stoppered

* The experiment described above can be perhaps even more easily carried out with any of the stable animal oils which are sold for lubricating fine machinery. Unstable oils, such as olive oil, are unsuitable, inasmuch as these last are broken up and converted into soaps when digested, as described above, with the barium hydrate solution.

bottle and is kept sheltered from light. Before it is employed for the purposes described below, it is sterilised by heating to 140° C. in a test-tube, and is each time re-tested by shaking up with the dilute sodium carbonate solution.

Procedure adopted for obtaining from the Finger a Sample of Blood without allowing this to come in contact with the External Air.

A receptacle for the blood is first provided by drawing out a test-tube to form such a "thimble" as is represented in fig. 5 (p. 66).

The thimble is filled in with sterilised fatty acid-free oil, and is covered in with a sterilised cover glass.

The ulnar aspect of a finger—preferably of the little finger of the left hand—is now sterilised by flaming alcohol. It is then punctured in two or three adjacent points by a fine spicule of glass. A clean handkerchief is wound round the digit, the tip of this last is immersed into the oil, and pressure is applied to the finger pulp. The blood as it emerges descends through the oil in the form of large globules.

When pressure on the pulp ceases to yield blood, the finger is momentarily removed from the oil, the handkerchief is loosened and re-applied, the finger is re-immersed into the oil, and pressure is again made on the finger pulp.

When a sufficiency of blood has been collected, a sterilised rubber test-tube cap is drawn over the thimble. This last is then placed in a hand-centrifuge, and the blood is, by a few turns of the handle, driven down to the lower narrow end of the tube.

After allowing an interval of 10 minutes to elapse, the centrifugalisation of the coagulated blood—the blood, it may be noted, invariably coagulates*—is taken in hand. The contents of the thimble will now arrange themselves into an upper layer of oil, a middle layer of clear serum, and a lower layer of blood corpuscles.

With a view to ensuring the asepticity of the further procedure, the serum may now with advantage be separated from the oil in the thimble, which has been exposed to some risk of aerial contamination.

Procedure for the Separation of the Serum from the Oil in the Thimble

The procedure is as follows :—

A series of three or four tubes, fig. 6, which are to function respectively as receiving and mixing tubes, are flamed, filled in with sterilised oil, horizontally inclined, and placed ready to hand. A capillary testing pipette, similar to that figured (fig. 4) and described

* (Added note.) The common text-book statement that coagulation is suspended when blood is collected under oil is, it may be presumed, based on experiments undertaken with oil containing free fatty acids. A decalcification of the blood might under such circumstances result.

n the first section of this paper, is fitted with a rubber teat; sterile oil is now aspirated into the pipette to replace the air in the capillary stem and the lower part of the bulb. When this replacement has been effected, the tip of the pipette is thrust down through the oil contained in the thimble into the layer of serum, and this last is aspirated into the pipette. When this has been accomplished, a little

FIG. 5.

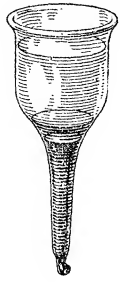
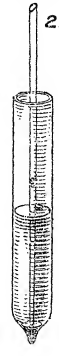


FIG. 6.



of the covering oil is drawn into the capillary tube to seal the lower orifice of the pipette. The serum, thus shut off from contact with the air, is carried across into the first of the receiving tubes already spoken of, and is driven out into this under cover of the oil.

Another sterile testing pipette is now taken in hand, and the procedure is repeated in all its details, the serum being carried across from the first into a second receiving tube.

Procedure Adopted for Measuring off Equal Volumes of the Serum and Culture, and Mixing these without Contact with the Air.

The next step is to mix the serum with the graduated dilutions of the bacterial culture. These dilutions will have been prepared* by diluting the culture with sterile broth, which has been previously boiled up in order to remove the contained air.

The procedure by which the serum and the culture are mixed is essentially the same as the procedure employed for transferring the serum from one vessel to another. The capillary pipette is first filled in with a sufficiency of sterile oil, secondly with serum up to the mark on the stem. Thirdly, a globule of oil is drawn in.

Employing this globule of oil as a seal for preventing contact with the air—it will presently function as an index globule—the pipette is

* They may, if desired, be prepared under oil and from cultures anaerobically grown under a covering layer of oil.

carried across to the vessel which contains the culture, and is filled in with this last up to the mark on the capillary stem.

Mixture of the serum and culture can now be effected either (*a*) in the pipette itself, or—and I owe this suggestion to my colleague, Major W. B. Leishman, R.A.M.C.,—(*b*) in a mixing tube such as is shown in fig. 6.

In the former case, oil in sufficient quantity to seal the lower end of the column of fluid is aspirated into the pipette, after the volumes of serum and culture have been measured in in the manner described. The contents of this tube are then cautiously drawn upwards until the walls of the capillary stem fall away sufficiently to liberate the index globule of oil. This obstacle having been got rid of, a series of upwards and downwards displacements of the combined column of fluid will bring about the desired mixture.

In the case where a mixing tube is employed, the extremity of the capillary pipette is carried down to the floor of the mixing tube, and the contents are driven out under the covering seal of oil. They are then intermixed by alternately drawing them in and driving them out of the capillary stem, care being taken that the pipette is never emptied of oil. Lastly, the mixed fluids are carefully and completely reaspirated from the floor of the narrowed end of the tube, the inflow being in each case allowed to continue until a sufficient seal of oil has been carried in behind the mixture of serum and culture. As soon as this has taken place, the point of the pipette is withdrawn from the oil, and air is allowed to enter and occupy the lower third of the stem. Finally, the orifice is sealed in the flame.

In filling in a succession of capillary pipettes from one and the same mixing tube, it will, as consideration will show, be advantageous to begin with the highest dilution, and to follow on in order with the lower dilutions.

Procedure for determining the Bactericidal Effect exerted by the Serum in the absence of Air.

The process of filling in the bulb of the capillary pipette with sterile nutrient broth—a process which is, in the case of the ordinary aerobic procedure described in the previous section, undertaken as a first step in the filling in of the pipette—is, in the case of the anaerobic procedure, undertaken as the final procedure after the serum and the culture have been in contact for the desired period.

It is carried out in the following way :—

Sterile nutrient broth having been placed ready in a covered watch-glass, the capillary pipette which contains the highest dilution of the culture is taken in hand. A negative pressure having been established in its interior by fitting on a collapsed rubber teat, the lower portion

of the stem is passed through the flame of a peep-light in such a way as to heat it without allowing it to fuse and to collapse under the influence of the internal negative pressure. The sterilised extremity is now snapped off by plunging it while still hot into the nutrient broth. The inflow which takes place through the orifice thus provided is arrested by the pressure of the finger and thumb upon the teat as soon as the cultivation chamber is about two-thirds full.

The sealing up of the tube and the subsequent cultivations are carried out in exactly the same manner as in the case of the ordinary (aerobic) estimation described in Section I.

SECTION III.—*On the Bactericidal Effects produced by one and the same Human Blood* (a) *Drawn off and Tested by the Aerobic Procedure described in Section I; and* (b) *Drawn off and Tested by the Anaerobic Procedure described in Section II.*

In view of the fundamental theoretical importance which attaches to the assumption that the bactericidal power of the blood is acquired only after withdrawal from the organism, and, in particular, after the disintegration of the leucocytes under the influence of air and contact with the wall of unoiled or unparaffined receptacles, it seemed important to reinvestigate the question; I have therefore endeavoured to ascertain whether there is any constant and important difference between the bactericidal power of human blood (*a*) drawn off and tested by the aerobic procedure described in Section I; and (*b*) drawn off and tested by the anaerobic procedure described in Section II.

The results of this investigation are set forth below in tabular form, and it will be observed that while they are, of course, inconclusive on the wider question of the derivation of the bactericidal substances of the serum, they would seem definitely to show that neither contact with the external air, nor contact with ordinary glass surfaces, exerts any important influence on the bactericidal power exerted by human blood upon the typhoid bacillus and the cholera vibrio.

Table exhibiting the Bactericidal Effect produced by one and the same Serum (*a*) drawn off and tested by the Aer

Dilutions in which the culture was employed.	1 vol. A. E. W.'s serum. 1 vol. of a typhoid culture, containing 156,000,000 T.B. per c.c.		1 vol. W. B. L.'s serum. 1 vol. of a typhoid culture, containing 156,000,000 T.B. per c.c.		1 vol. F. N. W.'s serum. 1 vol. of a typhoid culture, containing 156,000,000 T.B. per c.c.		1 vol. A. E. W.'s serum. 1 vol. of a typhoid culture, containing 120,000,000 T.B. per c.c.		Capilla 1 vol. F. 1 vol. c culture 150,000 c.c.
	Aerobic procedure.	Anaerobic procedure.	Aerobic procedure.	Anaerobic procedure.	Aerobic procedure.	Anaerobic procedure.	Aerobic procedure.	Anaerobic procedure.	Aerobic procedur
Undiluted.	—	—	—	—	—	—	—	—	Growth
2-fold dilution..	—	—	—	—	—	—	—	—	,,
5 ,, ,,	Sterile	Sterile	Growth	Sterile	Growth	Growth	Growth	Growth	,,
10 ,, ,,	,,	,,	Sterile	,,	,,	,,	,,	,,	,,
25 ,, ,,	,,	,,	,,	,,	,,	Sterile	,,	,,	,,
50 ,, ,,	,,	,,	,,	,,	,,	,,	Sterile	Sterile	Sterile
100 ,, ,,	,,	,,	,,	,,	,,	,,	Growth	,,	,,
1,000 ,, ,,	,,	,,	,,	,,	,,	,,	Sterile	,,	,,
10,000 ,, ,,	,,	,,	,,	,,	,,	,,	,,	,,	,,
100,000 ,, ,,	,,	,,	,,	,,	,,	,,	,,	,,	,,

A. E. W. had been inoculated against typhoid; W. B. L., F. N. W., and A. B, were normal men; J. N. had recently convalesced fron
The sera were in each case tested within 2—3 hours after the blood had been withdrawn. The culture were in all cases aerobically gro

the Aerobic Procedure described in Section I; and (*b*) drawn off and tested by the Anaerobic Procedure described in Secti

Capillary testing pipettes were filled in with —

1 vol. F. N. W.'s serum. 1 vol. of a typhoid culture, containing 150,000,000 T.B. per c.c.		1 vol. A. E. W.'s serum. 1 vol. of a typhoid culture, containing 100,000,000 T.B. per c.c.		1 vol. A. B.'s serum. 1 vol. of a typhoid culture, containing 220,000,000 T.B. per c.c.		1 vol. J. N.'s serum. 1 vol. of a typhoid culture, containing 540,000,000 T.B. per c.c.		1 vol. A. E. W.'s serum. 1 vol. of a cholera culture, containing 18,000,000 cholera vibrios per c.c.		1 vol. A. F 1 vol. o culture, 60,000,0 vibrios p
Aerobic procedure.	Anaerobic procedure.	Aerobic procedure.	Anaerobic procedure.	Aerobic procedure.	Anaerobic procedure.	Aerobic procedure.	Anaerobic procedure.	Aerobic procedure.	Anaerobic procedure.	Aerobic procedure.
Growth	Growth	—	—	—	—	—	—	Growth	Growth	Growth
,,	,,	Growth.	Growth	—	—	Growth	Growth	Sterile	Sterile	,,
,,	,,	,,	,,	—	—	Sterile	,,	,,	,,	,,
,,	,,	Sterile	,,	Growth	Growth	,,	,,	,,	,,	,,
,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,
Sterile	Sterile	,,	,,	Sterile	Sterile	,,	,,	,,	,,	Sterile
,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,
,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,
,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,
,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,

esced from typhoid.

bically grown 24-hours-old broth cultures. The serum was in each case allowed to act upon the culture for 18—24 hours at a temperature of 37

d in Section II.

1 vol. A. E. W.'s serum. 1 vol. of a cholera culture, containing 60,000,000 cholera vibrios per c.c.	
Aerobic procedure.	Anaerobic procedure.
Growth	Growth
,,	,,
,,	,,
,,	,,
,,	,,
Sterile	Sterile
,,	,,
,,	,,
,,	,,
,,	,,

ature of 37° C.

FIG. 1.

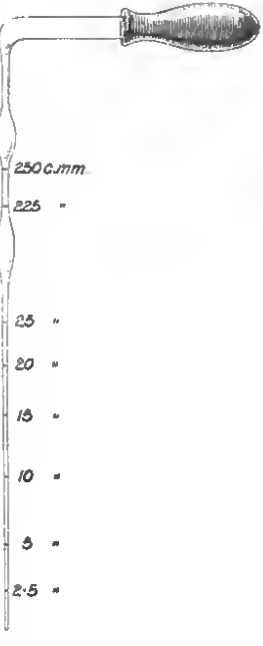


FIG 2

FIG. 3.

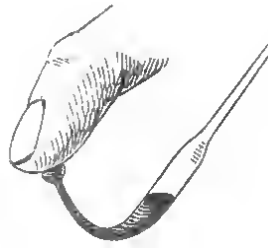


FIG. 5.

Fig. 6.

Table exhibiting the Bactericidal Effect produced by one and the same Serum (*a*) drawn off and tested by the Aerobic Procedure described in Section I, and (*b*) drawn off and tested by the Anaerobic Procedure described in Section II

Capillary testing pipettes were filled in with

Dilutions to which the cultures were employed.	1 vol. A F W serum + 1 vol. of a typhoid culture containing 240,000,000 T B per c.c.		1 vol. W N L serum + 1 vol. of a typhoid culture containing 480,000,000 T B per c.c.		1 vol. F K W serum + 1 vol. of a typhoid culture containing 480,000,000 T B per c.c.		1 vol. A. E. W serum + 1 vol. of a typhoid culture containing 120,000,000 T B. per c.c.		1 vol. F N W serum + 1 vol. of a typhoid culture containing 480,000,000 T B. per c.c.		1 vol. A. E. W serum + 1 vol. of a typhoid culture containing 120,000,000 T B. per c.c.		1 vol. A. B. serum + 1 vol. of a typhoid culture containing 240,000,000 T B per c.c.		1 vol. J N serum + 1 vol. of a typhoid culture, containing 240,000,000 T B per c.c.		1 vol. A F W serum + 1 vol. of a cholera culture containing 12,000,000 cholera vibrios per c.c.		1 vol. A. E. W serum + 1 vol. of a cholera culture containing 60,000,000 cholera vibrios per c.c.	
	Aerobic procedure	Anaerobic procedure	Aerobic procedure	Anaerobic procedure	Aerobic procedure	Anaerobic procedure.	Aerobic procedure	Anaerobic procedure	Aerobic procedure	Anaerobic procedure.	Aerobic procedure	Anaerobic procedure.	Aerobic procedure.	Anaerobic procedure.	Aerobic procedure	Anaerobic procedure	Aerobic procedure	Anaerobic procedure	Aerobic procedure.	Anaerobic procedure
Undiluted,	—	—	—	—	—	—	—	—	Growth	Growth	—	—	—	—	—	—	Growth	Growth	Growth	Growth
2-fold Dilution	—	—	—	—	—	—	—	—	Growth.	Growth	—	—	—	—	Growth	Growth	Sterile	Sterile	"	"
5	Sterile	Sterile	Growth	Sterile	Growth	Growth	Growth	Growth	"	"	—	—	Sterile	"	"	"	"	"	"	"
10	"		Sterile		"		"		"	"	Sterile	"	Growth	Growth	"	"	"	"	"	"
20	"	—	—	"	"	Sterile	"		"	"	—	—	—	—	"	"	"	"	"	"
40	"	—	—	—	"		Sterile	Sterile	Sterile	Sterile	—	—	Sterile	Sterile	"	"	"	"	Sterile	Sterile
100	"	—	—	"	—		Growth		"	"	"	"	"	"	"	"	"	"	"	"
2,000	"	—	"	"	—	"	Sterile		"	"	"	"	"	"	"	"	"	"	"	"
10 000		—	"	—	"	—	"		"	"	—	—	"	"	"	"	"	"	"	"
20 000		—	—	—	—	—	—		"	"	—	—	—	—	"	"	"	"	"	"

A F W had been neutralised against typhoid; W N L, F K W and A. B were normal sera; J N had recently spontaneously from typhoid.

The tests were in each case tested within 8—24 hours after the blood had been withdrawn. The cultures were in all cases accidentally grown 48-hours-old broth cultures. The serum was in each case allowed to act upon the culture for 18—24 hours at a temperature of 37° C.